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**PHYSICO-CHEMICAL CHARACTERIZATION AND APPLICATION OF PECTIN EXTRACTED FROM SEASONALLY AVAILABLE DIFFERENT FRUITS WASTE IN JELLY PREPARATION**

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Roll No: 0115/09

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**A thesis submitted in the partial fulfillment of the requirements for the**

**degree of Masters of Science in Applied Human Nutrition and Dietetics**

**Department of Applied Food Science and Nutrition**

**Faculty of Food Science and Technology**

**Chittagong Veterinary and Animal Sciences University**

**Chittagong-4225, Bangladesh**

**December, 2016**

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**December, 2016**

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**This is to certify that we have examined the above Master’s thesis and**

**have found that is complete and satisfactory in all respects, and that all**

**revisions required by the thesis examination committee have been made**

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#

# LIST OF ABBREVIATION

|  |  |
| --- | --- |
| AUA  | Anhydrouronic Acid |
| DE  | Degree of Esterification |
| DM  | Degree of Methylation |
| gm  | Gram |
| HCl  | Hydrochloric Acid |
| HM  | High Methoxy pectin |
| Kg  | Killo gram |
| LM  | Low Methoxy pectin |
| MeO  | Methoxyl content |
| mg  | Milli gram |
| Meq | Milli equivalent |
| ml  | Millilitre |
| NaCl | Sodium chloride |
| NaOH | Sodium Hydroxide |
| TSS  | Total Soluble Solid |

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# SUMMARY

Pectin is widely used as a gelling agent, thickener, emulsifier and stabilizer in different food processing operations. Chemically, it represents a polysaccharide, which is present in different amount in cell walls of all land plants. But some fruits are very rich in this component and can be used as source for its production commercially. In this study, mango peel, guava peel and jackfruit peel were selected as a source of pectin because of the abundance these fruits in Bangladesh. These peels were heated in a solution of distilled water and HCl at a temperature of 40-80°C for 10-40 minutes with continuous stirring. The solution was filtered out and the filtrate was treated with different low molecular weight alcohol such as ethanol to extract pectin. The precipitate was dried at 55°C under vacuum. The product was characterized by the parameters as methoxyl content, anhydrouronic acid content, degree of esterification, equivalent weight, ash and moisture. The characteristic parameters were found to be in the same range as those shown by the product proposed in the market as ‘Pectin’. Finally, this pectinwas used for the production of jelly.

**Keywords:** Extraction, Pectin, Yield, Methoxyl Content, Anhydrouronic acid, Degree of esterification, Jelly

# CHAPTER 1: INTRODUCTION

In Bangladesh, there are lots of food industries. These industries produce different types of foods. There are some food industries, those use fruits for food preparation and processing of different kinds of fruit juice, jam, jelly, chocolates, bar and so on. These industries mainly used seasonally available fruits. On the other hand, large volume of fruit wastes is produced from these food industries. Fruit wastes are highly perishable and seasonal. People of our country especially industrial people cannot properly handle fruit wastes, that’s why it’s a problem to the processing industries (Apsara & Pushpalatha, 2003).

These wastes lead to serious pollution problem in the environment and it also represent a loss of valuable nutrients and biomass (Chacko & Estherlydia, 2014). Environmental pollution problem can be reduced by by-product recovery from fruit wastes. It also improves the overall economy of the processing unit of a food processing industry. Pectin is a valuable by-product which can be extracted from fruit wastes. Pectin is an important element which is found in the cell wall of the fruit. If food industries use the fruit peel for the extraction of pectin, then it will reduce the pollution problem.

The main things about pectin is it has a good gelling property, which is used in different food preparation and many more things (Apsara & Pushpalatha, 2003). Pectin is used in manufacture of jams, jellies, marmalades, preserves etc. It is also useful as a thickening agent for sauces, ketchups, flavored syrups and as a texture agent in fruit-flavored milk deserts. Besides, it has numerous applications in pharmaceutical preparations, pastes, cosmetics etc. But, the single largest use of pectin is in the manufacture of jellies. Most of the commercial pectin in the world is used to make jelly and similar products.

In our country, most of the jam or jelly production industries use commercial pectin. These industries brought pectin from other countries to produce good quality of product. Thus, it also increases the production cost. From our current understanding, we found this one as a gap in our food industry which need to be addresses. In our study, we extracted pectin from our seasonally available low cost fruit wastes and used that pectin to prepare jelly. The present study was therefore designed to estimate pectin from seasonally available fruit wastes.

**The objectives of this study are as follows**

1. To extract pectin as stabilizers, gelling agent and thickening agent from locally available low cost fruits waste.
2. To characterize extracted pectin.
3. To apply isolated pectin in jelly preparation.
4. To compare the functional quality of jelly.

# CHAPTER 2: REVIEW OF LITERATURE

Fruit wastes can contain many reusable substances of high value. These reusable substances can be converted into commercial product or a new product. A very little study has so far been made on fruit waste processing, preservation and storage in Bangladesh to prevent seasonal glut and to ensure the availability of this favorite fruit round the year. A review of the available information relating to the present study is given here.

## 2.1 Guava

Guava (*Psidiumguava*) is locally known as “**Peara”** and belongs to the family “**Myrtaceae”**. It grows abundantly in Bangladesh, India and in many parts of Southeast Asia (Islam *et al*., 2011). Guava is a good source of vitamin C. For the synthesis of collagen and neurotransmitter vitamin C is an effective anti-oxidant. In addition, vitamin C is important for the synthesis of carnitine that is essential for the transport of fat to mitochondria for conversion to energy (Carr & Frei, 1999). Guava contains some important nutrients like carbohydrate, protein, fat, iron, sodium and potassium (Haque *et al*., 2009).

## 2.2 Mango

The scientific name of mango is *Mangiferaindica* and it belongs to the family **“Anacardiaceae”.** It is locally known as “**Aam”** (Islam *et al*., 2011). Mango is cultivated commercially in many countries which are the Southeast Asia, namely, India, Pakistan, the Philippines, Indonesia, Malaysia, Thailand, Burma, Sri Lanka, and Bangladesh. It is a fruit crop for home consumption. Nutritionally, it contains important elements which are 𝛽-carotene, vitamin C, and dietary fiber. It also contains soluble sugars and different minerals, which are readily available and easily assumable in human body and therefore it is capable to prevent many deficiency diseases (Khan *et al*., 2013).

## 2.3 Jackfruit

Jackfruit (*Artocarpusheterophyllus Lam.*) trees belong to the family **“Moraceae”.** It is locally known as **“Kathal”**. They grow abundantly in India, Bangladesh, and in many parts of Southeast Asia. It is considered as one the most significant evergreen trees in tropical areas and widely grown in Asia including India. Jackfruit is a good source of protein, starch, calcium, and thiamine (Swami *et al*., 2012).

## 2.4 Pectin

All fruit peels contain pectin at their cell wall and some pectin is also found in fruit pulp. Pectin is an important factor during fruit ripening, and the amount of pectin varies in different types of fruits. Pectin is derived from heteropolysaccharides, which is extracted from the primary cell wall of higher plants. Pectin is a functional ingredient in the food industry because it has a good gelling ability and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products. Also, the pharmaceutical industries widely use pectin. It has been reported that, pectin lower the blood cholesterol levels and low density lipoprotein cholesterol fractions, which is beneficial for human health. It is also stated that, pectin may help decrease tumor cell formation (Bhat *et al*., 2014). Pectin can also be used in several ways like biodegradable water-soluble films, bulking agents, coating agents, chelators, emulsifiers and viscosity modifiers (Kanmani *et al*., 2014).

### 2.4.1 General properties of pectin

Pectin’s are soluble in pure water. Monovalent cation salts which are pectinic and pectic acids are usually soluble in water; di and trivalent cations salts are weakly soluble or insoluble. When dry powdered pectin’s are added to water it hydrates very rapidly, forming clumps. These clumps are of semidry packets of pectin contained in an envelope of highly hydrated outer coating. Clump formation can be prevented by dry mixing pectin powder with water-soluble carrier material (Whistler and BeMiller,1993).

The nature of dilute pectin solutions is Newtonian but at a moderate concentration, they exhibit the non-Newtonian, pseudo plastic behavior. Viscosity, solubility, and gelation are generally related with these. For example, factors that increase gel strength will increase the tendency of gel formation, decrease solubility, and increase viscosity. These properties are the function of pectin’s structure, which is that of a linear poly anion. Such as, in solution some monovalent cation salts of pectin’s are highly ionized, and the distribution of ionic charges in the molecule tends to keep it in an extended form by coulombic repulsion (Paoletti, 1986).

This same coulombic repulsion act between the carboxylate anions to prevent aggregation of the polymer chains. In addition, each polysaccharide chain, especially each carboxylate group, will be highly hydrated. Monovalent salts of pectin’s solution exhibit stable viscosity because each polymer chain is hydrated, extended, and independent. If the pH is lowered then ionization of the carboxylate groups is decreased, and this results in a reduction in hydration of the carboxylic acid groups. As a result of reduced ionization, the polysaccharide molecules no longer repel each other, that’s why they can associate and form a gel. Apparent pK values vary with the DE of the pectin (Plashchina et al., 1978); a 65% DE pectin has an apparent pK of 3.55, while a 0% DE pectic acid has an apparent pK of 4.10.

The main use of pectin is based on its ability to form gels. Pectin forms gels with sugar and acid. Gel is formed by hydrogen bonding between free carboxyl groups on the pectin molecules and between the hydroxyl groups of neighboring molecules. In a neutral or only slightly acid dispersion of pectin molecules, most of the un-esterified carboxyl groups are present as partially ionized salts. When acid is added then the carboxyl ions are converted to mostly unionized carboxylic acid groups. This reduce the number of negative charges not only lowers the attraction between pectin and water molecules, but also lowers the repulsion between pectin molecules. For competing water sugar further decreases hydration of the pectin. The degree of esterification is also affected by the rate of gel formation. Rapid setting of gel is caused by the higher DE. Rapid-set pectin’s (i.e. pectin with a DE of above 72%) also gel at lower soluble solids and higher levels than slow-set pectin’s (i.e. pectin with a DE of 58- 65%) (Raj *et al*., 2012).

Pectin has also applications in the pharmaceutical industry. Pectin reduces cholesterol levels in blood (Sriamornsak, 2001). Consumption of at least 6 g/day of pectin is necessary to have a significant effect in cholesterol reduction. Amounts less than 6 g/day of pectin are not effective for cholesterol reduction (Ginter *et al*.,1979).

### 2.4.2 Backbone structure

Pectin’s are a family of complex polysaccharides which contain 1,4-linked α-D-galactosyluronic residues. There are three pectic polysaccharides, these are homogalacturonan, rhamnogalacturonan-I and substituted galacturonans, isolated from primary plant cell walls. Homogalacturonan (HG) is a linear chain of 1,4-linked α-D-galactosyluronic residues, there are some of the carboxyl groups are methyl esterified. At the C-2 and C-3 positions they may be acetylated. Homogalacturonans have been isolated from sunflower heads and apple pectin but the extraction treatments were likely to cleave covalent bonds, that’s why it released from a heterogeneous pecticpolysaccharide (Sharma *et al*.,2006).



**Figure 1: Structure of pectin**

Commercial pectin’s are formed by a specific group of carbohydrate polymers which are composed of large backbone of linked D-galacturonic acid units, in that many of them are esterified with methyl alcohol at the carboxylic acid, interspersed with a few L-rhamnose residues linked to neutral arabinogalactan side chains. The most important application of pectin is in jellies with high sugar content, but it is also used in the pharmaceutical, dental and cosmetic industries for its jelling properties (Endress,1991).

###

### 2.4.3 Functional group

Pectin carries some non-sugar substituent’s, like methanol, acetic acid, phenolic acids and sometimes amide groups. Esterification of galacturonic acid residues with methanol is a very important structural characteristic of pectic substances. The definition of degree of methylation (DM) is the percentage of carbonyl groups esterified with methanol. Degree of methylation (DM) are two types, these are high-methoxy pectin (HM) and low-methoxy pectin (LM). More than 50% of the carboxyl groups are methylated and this pectin’s are called high-methoxy pectin’s (HM), on the other hand less than that degree of methylation are called low methoxy (LM) pectin’s. This same condition is applicable to acetylation. Acetyl groups can be found in the 'hairy' rhamnogalacturonan regions and only present in very low amount in homogalacturonan from apple and citrus. It is found in higher amounts in homogalacturonan from sugar beet and potato (Sharma *et al*.,2006).

### 2.4.4 Types of pectin

Based on formation of gel in the presence of divalent cations, sugar or acid, pectin’s can be classified into two groups:

#### 2.4.4.1 Low methoxy pectin (LM)

LM pectin form gel in the presence of divalent cations, usually calcium. Gelation is caused by the formation of intermolecular junction zones between homogalacturonic regions of different chains. The structure of such a junction zone is generally known as “egg box” binding process. Initial strong association of two polymers into a dimer form weak inter dimer aggregation, mainly regulated by electrostatic interactions. Gelation ability of LM pectin increases with decreasing degree of methylation. The presence of acetyl groups in pectin is very useful because it prevents gel formation with calcium ions and gives the pectin emulsion stabilizing properties (Ahmmed, 2013).

#### 2.4.4.2 High methoxy pectin (HM)

HM pectin’s can form gel with sugar and acid. This kind of gel is considered a 2-dimensional network of pectin molecules in which the water with sugar and acid are immobilized. The formation of the 3D network is based on the structure of junction zones in which there are chain associations stabilized by hydrogen bonding between un-dissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters. The gelation mechanism of pectin’s is mainly followed by their degree of esterification (DE). The attraction of pectin chains towards calcium is known to increase with decreasing degree of esterification, and with increasing polymer concentration. The influence of the charge density in polygalacturonate chain, and the distribution pattern of free and esterified carboxyl groups has an important effect on the strength of calcium binding. Molecular weight of pectin varies with plant source, raw material and extraction conditions but molecular weight determination is a challenge because of heterogeneity and aggregation which can bemist data gathering (Ahmmed, 2013). Some factors are responsible for the conditions of gel formation and the gel strength achieved. The major role of pectin molecules is that their chain length, and the chemical nature in the junction zones. At same conditions gel strengths increase with the molecular weight of the pectin, and any treatment de-polymerization of the pectin chains is reflected in weaker gel (Sharma & Naresh, 2006).

### 2.4.5 Sources of pectin

Most commonly pectin present in most of the plant tissues as a layer in the middle lamella and as a thickening on the cell wall. Pectin from different sources may be used for the commercial manufacture of pectin but in a limited amount. On the other hand, the pectin from different sources does not have the same gelling ability due to variations in molecular size (Simpson *et al*.,1984). Now a days, commercially acceptable pectin’s source are citrus peel and apple pomace. They produce different quality pectin’s, which are used for specific applications (May, 1990).

**Table 1: Pectin Content of Some Fruits (Renard et al., 1993)**

|  |  |
| --- | --- |
| **Fruit** | **% Pectin substance (Wet weight)** |
| Apple (*Malus spp*) | 0.5-1.6 |
| Apple pomace | 1.5-2.5 |
| Banana (*Musa acuminate* L) | 0.7-1.2 |
| Beet pulp *(Beta vulgaris)* | 1.0 |
| Carambola *(Averrhoa carambola)* | 0.66 |
| Carrot *(Daucuscarota)* | 0.2-0.5 |
| Giant granadilla *(Passifloraquandrangularis*L) | 0.4 |
| Guava *(Psidiumguajava*L.) | 0.77-0.99 |
| Lemon pulp *(Citrus limon)* | 2.5-4.0 |
| Lychee *(Litchi chinesis*S.) | 0.42 |
| Mango *(Mangiferaindica*L.) | 0.26-0.42 |
| Orange peel *(C. sinesis)* | 3.5-5.5 |
| Papaya *(Carcia papaya)* | 0.66-1.0 |
| Passion fruit *(Passiflora edulis* S.) | 0.5 |
| Passion fruit rind | 2.1-3.0 |
| Peaches *(Prunus persica)* | 0.1-0.9 |
| Pineapple (Ananas*comosus*L**.)** | 0.04-0.13 |
| Strawberries *(Fragariaananassa)* | 0.6-0.7 |
| Tamarind *(Tamarindusindica*L.) | 1.71 |
| Thimbleberry *(Rubusrosalfolius)* | 0.72 |
| Tomato fruit *(Lycopersiconesculentum)* | 0.2-0.6 |

### 2.4.6 Uses of pectin

Pectin can be used in many ways. It is used mainly in foods as a gelling agent, thickener, texterizer, emulsifier, and stabilizer. Recently, pectin has been used in low-calorie foods as a fat or sugar replacer. Different uses of pectin in food and other industries are discussed here:

#### 2.4.6.1 Jams and jellies

The major food items in which large amount of pectin’s are used are jam and jelly. Jam preparation requires brief cooking of the fruit to liberate juice and pectin through conversion of proto pectin to soluble pectin. Depending upon the conditions, additional pectin’s may be added at any point during preparation. Pectin is added as a dry powder mixed with sugar in a solution (Towel *et al.,* 1959).

#### 2.4.6.2 Conserves

Conserves are products that do not contain a sweetener but fruit juice or fruit concentrate contain sweetener. That’s why, their soluble solid contents are lower than the products containing sweetener. As they do not contain any added sugar, They highly preferable by consumers. The total soluble solid content of conserves is 55 to 62%. At the upper level, a rapid-set HM pectin is used, while at the lower level, a LM pectin is added to give the desired mouth feel and body of the products (Towel *et al.,* 1959).

#### 2.4.6.3 Bakers' jellies

Pectin is used to make jellies that are applied to prepare bakery products. HM pectin’s are thermally stable, is used to make jellies that are placed in the batter or dough and baked product. Fiber entanglements will further emphasize the gel structure if the fiber content is increased. LM pectin has a wide application in bakery jam and jelly production. The use of LM pectin use requires a large amount of pectin in the formula, compared with HM pectin, to the exact firmness (Hoefler, 1991).

#### 2.4.6.4 Confectionery products

Different flavored candies are produced using HM pectin. Artificial cherries can be made using pectin, where a synthetic medium is produce to control setting conditions (Peschardt, 1956)**.**Pectin is also used in edible coatings for inhibiting lipid migration in confectionery products (Brake *et al.,* 1993).

#### 2.4.6.5 Frozen barriers

Pectin is used in frozen foods to prevent crystal growth, syrup during thawing, and to improve shape (Buren, 1983). Ice-cream factories use pectin for ice-cream production. They use LM pectin’s to improve the texture and quality of ice creams (Decker, 1951). Pectin helps to improve the texture of frozen foods by controlling the ice crystal size in them. Pectin is also used in the different gelled pudding desserts, where the mixing of fruit syrup containing pectin with cold milk. This types of dessert can be prepared without refrigeration because of the use of pectin (Hoefler, 1991).

#### 2.4.6.6 Beverages

In recent years, fruit juice does not use the added sweeteners like sucrose, high fructose corn syrup or both. That’s why certain mouth feel is not present in the conventional soft drinks. The loss of mouth feel can be restored by the addition of HM pectin 0.05 to 0.10%. Pectin can also be used as a beverage-clouding agent (Hoefler, 1991; El-Shamei and El-Zoghbi, 1994).

#### 2.4.6.7 Barbecue sauce

LM is added to the barbecue sauces due to its flavor release attributes and texture. The LM pectin and calcium content in the mixture determines the product's final consistency and texture (Hoefler, 1991).

#### 2.4.6.8 Pharmaceutical uses

Food pectin is also used in pharmaceutical industry. Pectin influences cholesterol levels in blood and act against toxic cations. It is effective in removing lead and mercury from the gastrointestinal organs and respiratory tracts (Kohn,1982). When pectin is injected intravenously, it reduces the coagulation time of drawn blood, thus it is useful in controlling hemorrhage or local bleeding (Joseph,1956). On the other hand, pectin sulfate prolongs clotting time and can be used in place of heparin (Ramaswany *et al.,*1992).

For the treatment of iron deficiency anemia degraded pectin iron is used. Pectin has been reported to reduce blood cholesterol in a wide variety of subjects (Cedra *et al*., 1988). Cholesterol reduction in blood requires consumption of at least 6 g/day of pectin. If the amounts less than 6 g/day are not effective (Delbarre*et al*., 1977; Raymond*et al.,* 1977). The mixture of LM pectin, aluminum hydroxide, and magnesium oxide are very useful in the treatment of gastric and duodenal ulcers. Pectin alone or in combination with gelatin is used as an encapsulating agent for the preparation of medicine. HM pectin is used for the release of aspirin and act as a demulcent in minimizing the gastrointestinal (CIBA, 1967; Ashford *et al.*, 1994; Bender, 1970).

### 2.4.7 Nutritional aspects of pectin

#### 2.4.7.1 Source of dietary fiber

Pectin is collected from plant cell walls and is analyzed as a soluble and insoluble fraction in the form of galacturonic acid after hydrolysis. Those fruits and vegetables which are rich in pectin have dietary fiber contents in the range of 1-2%. Pectin fibers has higher hydration properties than other fibers and this property is used in different food production, for example in bakery products. It has been reported that replacement of flour with citrus fibers, apple flakes and concentrates in bakery and confectionery products had a positive sensory effect. Adsorbent and bulk-forming properties of pectin have been promoted in some multi-ingredient anti constipation and anti diarrhoeal preparations.

#### 2.4.7.2 Mineral binding

Dietary fiber can absorb and exchange mineral and ion. Pectin has the ability to associate ions Due to high content of negative charges and calcium binding pectin has the ability to associate with ions.

#### 2.4.7.3 Prebiotic effect

Another functionality of pectin is in prebiotic effect on human body. Pectin fermentation takes place in the large intestine by the action of bacteria. Pectin substituents are fermented in the colon by the formation of short- chain fatty acids. It has been reported that non-methyl-esterified pectin's were more rapidly fermented than methyl-esterified pectin’s. The end products of fermentation of pectin are the short-chain fatty acids, acetate, propionate and butyrate, as well as hydrogen and carbon dioxide. The short-chain fatty acids escape colonic metabolism and transported via the portal circulation to the liver where they undergo metabolism. In the liver, they enter the systemic circulation and are distributed to the various tissues of the body.

#### 2.4.7.4 Cholesterol regulation

Cholesterol regulation by pectin depends on the viscosity of pectin. Preparations of pectin with high viscosity appear to be more effective in lowering cholesterol than lower viscosity. High viscosity of pectin lower cholesterol levels by raising the excretion of fecal bile acids and neutral sterols. High-viscosity pectin may incorporate with the formation of micelle and lower the diffusion rate of bile acid and cholesterol-containing micelles through the bolus, consequently diminishing the uptake of cholesterol and bile acids. Pectin has also favorable effects on lipids.

## 2.5 Jelly

Jelly refers to a clear and transparent food product prepared from fruit juice with or without added pectin. Some fruit contain pectin in nature. That’s why in some jellies may not require pectin. Jelly processing can be same as jam processing. Exception is that in jam processing fruit pulp is used and in jelly filtered fresh fruit juice used. Good quality of jelly is clear, transparent and a fresh flavor of the fruit juice from which it is made. It has been reported that, the optimum pH for gel formation is 3.2. Beyond pH 3.2, that at pH 3.4 gel formation occurs at the usable soluble solid range. And the optimum sugar concentration for gel formation is 67.5%. Above this level sticky consistency of jelly form, and lower the level form weak jelly (Desrosier, 1963).

# CHAPTER 3: MATERIALS & METHODS

## 3.1 Sample collection

The fruit samples were collected from local market of Chittagong district.

## 3.2 Experimental design

The schematic representation of experimental design is presented in Figure 2.

**Figure 2: Experimental design of the study**

## 3.3 Study period

The study was conducted for a period of two months, starting from April 2016 - June 2016.

## 3.4 Sample size

Total 3 types of fruit samples were collected from the local market of Chittagong.

## 3.5 Instruments and reagents

Some instruments and reagents were used during this study period, these are -

### 3.5.1 Instruments

* Weight balance- VA120
* Digital pH meter- JENWAY
* Hot air oven- Presto’s hot air oven
* Muffle furnace- BIONICS
* Glassware ( beaker, funnel, separating funnel, glass rod, conical flask)
* Filter paper- Whatman filter paper
* Refractometer- Milato
* Utensils ( knife, chopping board, pan, spoon, electric oven)
* Burette stand
* Electric heater- AREC heating machine
* Magnetic stirrer- AREC heating magnetic stirrer
* Nylon cloth- 100 micron
* Dessicator- Vacuum Dessicator
* Crucible

###

### 3.5.2 Reagents

* 96% Ethanol
* Distilled water
* NaOH
* NaCl
* HCl
* Phenol red indicator
* Citric acid (Lime juice)

## 3.6 Methods

### 3.6.1 Collection of fruit waste sample

At first three different types of fruits are collected from local market of Chittagong. Then the fruits are washed with water to remove dust or any other particles. After that fruit peels and the waste are collected from the fruits placed in a clean sampling bag and stored in a freezer prior to pectin extraction to prevent breakdown of the peels and microbial attack.

### 3.6.2 Extraction of pectin from Mango peel

The mango peels were subjected to hydrolysis by treating the raw materials with distilled water acidified at different pH levels (1.5, 1.8, 2.5), temperature 40-80°C and heating time 10-40 minutes with continuous stirring to establish the extraction condition that will give the highest pectin yield. The resulting extract was cooled to 25°C and filtered to remove solids/insoluble residues to obtain the pectic liquor.

In a separating funnel the pectic liquor was taken with equal volume of 96 percent ethyl alcohol. The mixture was left 1hr to allow the pectin float on the surface. The gelatinous pectin flocculants then skimmed off.

The coagulated pectin purified by washing 100ml acetone and then pressed on nylon cloth to remove the residual acid and universal salt recovered from the liquor by passing this through silk cloth.

The resulted pectic substance dried at 55°C in an air forced oven. Percentage yield of pectin from initial peels can determined on both wet and dry weight basis.

 % Pectin recovery = Weight of pectin x 100

 Weight of mango peel

### 3.6.3 Extraction of pectin from Guava peel

The guava peels were subjected to hydrolysis by treating the raw materials with distilled water acidified at different pH levels (1.9,2.0, 2.5), temperature 40-80°C and heating time 10-40 minutes with continuous stirring to establish the extraction condition that will give the highest pectin yield. The resulting extract was cooled to 25°C and filtered to remove solids/insoluble residues to obtain the pectic liquor. After collecting the pectic liquor the next procedures is same as 3.5.2.

### 3.6.4 Extraction of pectin from jackfruit waste

The jackfruit rind and core were subjected to hydrolysis by treating the raw materials with distilled water acidified at different pH levels (2.2, 2.5, 2.6), temperature 40-80°C and heating time 10-40 minutes with continuous stirring to establish the extraction condition that will give the highest pectin yield. The resulting extract was cooled to 25°C and filtered to remove solids/insoluble residues to obtain the pectic liquor. After collectingthe pectic liquor the next procedures is same as 3.5.2.

###

### 3.6.5 Physio-chemical characterization of pectin sample

The dried pectin samples obtained from three types of fruit waste were subjected to the following qualitative and quantitative test in order to characterize them.

#### 3.6.5.1 Qualitative tests

##### 3.6.5.1.1 Color

This was done by visual observation.

##### 3.6.5.1.2 Solubility of dry pectin in cold and hot water

0.25g pectin samples were taken in two conical flasks separately, then 10 mL of 95% ethanol and 50 mL of distilled water were added in these two flasks. The mixture of the second flask was shaken vigorously to form a suspension which was then heated at 85-95°C for 15min (Fishman *et al.*, 2003).

##### 3.6.5.1.3 Solubility of pectin solution in cold and hot alkali

1 mL of 0.1N NaOH was placed in two different conical flasks, 5ml of pectin solution was added and the second flask was heated at 85- 90°C for 15 min (Joslyn, 1980).

#### 3.6.5.2 Quantitative tests

##### 3.6.5.2.1 Equivalent weight (Titration A)

0.5g pectin sample was weighed into a 250mL conical flask and moistened with 5 mL ethanol. 1.0 g NaCl was added in this mixture followed by 100 mL distilled water and few drops of phenol red indicator. It should be noted that all the pectin had dissolved and no clumping occurred. The solution was slowly titrated with 0.1 M NaOH to the end point of pale permanent pink color (Plashchina *et al.,* 1978). Equivalent weight was calculated using the following formula -

 (*Weight of pectin sample × Molarity of alkali) × 100*

*Equivalent Weight =*

 *Volume of alkali*

##### 3.6.5.2.3 Methoxyl content (MeO) (Titration B)

This was done by the solution obtained from equivalent weight determination. 25 mL of 0.25M NaOH was added to the solution and the mixture was stirred thoroughly and allowed to stand for 30 min at ambient temperature. 25 ml of 0.25N HCl was added and titrated with 0.1N NaOH to the same end point as earlier (Kanmani *et al*., 2014).

The percentage of methoxyl content was calculated using the following formula

 *Volume of alkali × Weight*

*Methoxyl content % = × 100*

*Weight of pectin sample*

##### 3.6.5.2.4 Moisture content

An empty crucible was dried in an oven, cooled in a desiccator and its weight was measured. 5 g of pectin sample was taken to it and placed in a hot air oven at 100°C for 1 h. After that the crucible was removed, cooled in a desiccator and weighed (Kanmani *et al*., 2014). This process was repeated for three times. The moisture content was calculated using the following formula -

 *Weight of the Residue* × 100

*Moisture content %=*

 *Weight of the sample*

##### 3.6.5.2.5 Anhydrouronic acid (AUA) content

The AUA content was calculated using the values of equivalent weight and methoxyl content determined earlier, according to that formula (Owens *et al.,* 1952).

 176 × 100

 AUA % =

 Z

Where, 176 is the molecular weight of AUA and

 *Weight of sample(mg)*

*Z =*

 *meq of Titration A +meq of Titration B*

##### 3.6.5.2.6 Degree of esterification (DE)

The DE of pectin was calculated using the data from methoxyl and anhydrouronic acid content determinations (Schultz, 1976).

DE (%) = 176× MeO×100

 31×AUA%

##### 3.6.5.2.7 Ash content

0.5 g of pectin sample was taken in a dry, clean crucible and weighed accurately. At first moisture was removed by the hot air oven method. Then the sample was burnt in the muffle furnace under higher temperature. Then sample was placed into the muffle furnace and burnt for 4 to 6 hours at a temperature of 550°C and ignited until light gray color formed. The sample with crucible was then cooled in a desiccator and weighed (Ahmmed, 2013). The ash content was determined by using the following formula

Ash (%) = weight of residue/weight of sample× 100

## 3.7 Preparation of jelly

### 3.7.1 Sample collection

Three types of fruits were collected from local market of Chittagong. These fruits were pineapple, black- berry and star fruit.

### 3.7.2 Study design

The schematic representation of experimental design is presented in following

**Figure 3: Flow-sheet of jelly processing**

### 3.7.3 Preparation of Star fruit jelly

For preparation of Star fruit jelly, firm but not over ripe star fruits were chosen for jelly preparation. At first fruits were washed with water for the removal of dust or any other foreign particles. Fruits were cutting into thin slices. These thin slices were boiled with water for about 20-30minutes. Citric Acid (Lime juice) were added during boiling for preservation of jelly. The extract was strained through a strainer. Then the pectin which was collected from guava peel was added to the extract and the sugar. Again, the solution was boiled. The end-point of the jelly was judge bye sheet or drop test. Scum or foam which was produced during boiling removed from the boiling pan. Jelly was filled hot into a clean sterilized bottle and followed by capping. Then stored at ambient temperature.

###

### 3.7.4 Preparation of pineapple jelly

For preparation of pineapple jelly fresh ripe pineapples were chosen. Pineapples were washed with water for removal of dust or other particles. Then fruit peels were removed by a knife and cut into thin slices. After that the next procedure was same as 3.6.3. But in this jelly the pectin used which was extracted from mango peel.

###

### 3.7.5 Preparation of black-berry jelly

For preparation of Black-berry jelly fresh berries were collected. In that case, the berries were boiled after washing with water. Then the process is same as 3.6.3. And the pectin used which was extracted from jackfruit pectin.

##

## 3.8 Chemical analysis of jelly

### 3.8.1 Moisture content determination

Oven drying method were applied for the moisture content determination of jelly. At first 5g sample was taken in a clean dry crucible and placed in an oven for 72 hours at 80°C (Sarower*,* 2013). Moisture content was determined using the following formula

Moisture%=w1-w2/w1×100

Where,

w1= Initial weight of sample

w2= Final weight of sample

### 3.8.2 Ash content determination

For ash content determination 2 g of sample was taken in dry, clean crucible. At first moisture was removed using hot air oven method. Then the sample was burnt on an electrical heater. It was done to avoid the loss of sample in the muffle furnace under higher temperature. Then the sample was placed into the muffle furnace and burnt at 550°C temperature for 4-6 hours and ignited. After that it was then cooled in desiccators and weight was measured (Sarower*,* 2013). The ash content was calculated as follows

 Ash%=weight of residue/weight of sample ×100

### 3.8.3 Total soluble solid content determination

Total soluble solid content was determined using the refractometer. Two drops of jelly were placed on the refractometer prism and the prism was closed carefully. Make sure the sample was spreads evenly over the surface of the prism. The refractometer was hold near a source of light and looked through the end piece. The line between the dark and light fields was seen then the total soluble solids of the samples were read directly from the refractometer (Sarower*,* 2013).

###

### 3.8.4 Active acidity(pH) determination

The pH of the juice was measured by using digital pH meter at an ambient temperature.

##

## 3.9 Functional quality of jelly

The functional quality of jelly mainly examined by visual judgment.

###

### 3.9.1 Rate of setting and setting time

When jelly was poured into a glass jar then it was examined for its setting time. Setting time was recorded based on setting of jelly (Apsara & Pushpalatha, 2003).

### 3.9.2 Consistency

After setting of a jelly it was examined for its consistency such as gel, firm or syrupy. If water separate from a jelly, then it was denoted as weeping jelly. Crystal formation also observed in this test (Apsara & Pushpalatha, 2003).

### 3.9.3 Cloudiness

Absence of clarity in jelly was observed. If clarity was not observed in jelly, then it was denoted as cloudy jelly. And if cloudiness was not observed then it was denoted as clear, transparent jelly (Apsara & Pushpalatha, 2003).

#

# CHAPTER 4: RESULTS & DISCUSSION

## 4.1 Pectin recovery

Recovery of pectin from three different fruit peels were different. These fruit peels were trialed three times. The average pectin yield percentage were presented in the table 2.

**Table 2: Percent yield of pectin from guava peel, mango peel and jackfruit peel**

|  |  |
| --- | --- |
| **Sample** | **Yield (%)** |
| Guava peel | 13.44 |
| Mango peel | 8.26 |
| Jackfruit peel | 5.94 |

## 4.2 Characterization of pectin

The characterization of powdered pectin obtained from guava peel, mango peel and jackfruit waste were carried out for various parameters to evaluate its suitability in food systems. The moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid and degree of esterification given in the following table.

**Table 3: Chemical properties of extracted pectin**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Moisture****(%)** | **Ash (%)** | **Equivalent weight(gm/mol)** | **MeO(%)** | **AUA (%)** | **DE (%)** |
| **Guava peel** | 6.4 | 1.44 |  548.24 |  3.42 | 53.92 | 54.24 |
| **Mango Peel** | 7.1 | 4.69 |  634.88 |  6.25 | 53.22 | 53.33 |
| **Jackfruit Peel** | 8.24 | 5.92 |  460.8 | 3.36 | 43.52 | 35.94 |

###

### 4.2.1 Color

The color of guava, mango and jackfruit pectin color were whitish to light brown, grayish to light brown and light brown respectively. The color of pectin was performed by visual inspection.

### 4.2.2 Moisture content

The moisture content of guava pectin was 6.4%. The result was less than 12.44% (Bhat *et al*., 2014). The moisture content of mango pectin was 7.1% which was similar to the moisture content of mango pectin 8.875% (Lokhande *et al*.,2016). Shaha *et al.,* (2013) reported that the moisture content of pectin of papaya was 8.58%, Assam lemon was 7.08% and jackfruit was 10.2%. The moisture content of jackfruit pectin was 8.24%, close to the reported values.

### 4.2.3 Ash

The ash content of the guava pectin was 1.44% that was close to the reported value 1.8% (Bhat *et al*., 2014). The ash content of mango pectin and jackfruit pectin were 4.69% and 5.92%. It was reported that the ash content of mango pectin was 5.864%, which was similar to the extracted pectin ash content. On the other hand, the ash content of reported jackfruit pectin was 7.40% (Lokhande *et al*.,2016).

###

### 4.2.4 Equivalent weight

The equivalent weight of extracted pectin was 548.24 in guava pectin which was lower than 735-833 (Shaha *et al.,* 2013). The equivalent weight of mango pectin was 634.88, which was related to793.6 (Lokhande *et al*.,2016) Lastly, the equivalent weight of jackfruit pectin was 460.8 and the reported value was 576 (Lokhande *et al*.,2016).

###

### 4.2.5 Methoxyl content

The methoxyl content of extracted guava peel pectin was 3.42%, whereas the reported value was 4.25% (Bhat *et al*., 2014). Methoxyl content value of mango and jackfruit pectin were 6.25% and 3.36%. Which were similar to the reported value 7.812% and 4.20% (Lokhande *et al*.,2016).

###

### 4.2.6 Anhydrouronic acid content

The purity of pectin is determined by acid the Anhydrouronic (AUA) content and it is suggested that it should not be less than 65% (Bhat *et al*., 2014). The AUA of extracted guava, mango and jackfruit pectin were53.92%, 53.22%, 43.22% which were lower than the reported value.

### 4.2.7 Degree of esterification

The degree of esterification of extracted guava, mango and jackfruit pectin were 54.24%, 53.33% and 35.94%. From the above result guava and mango pectin were the HM pectin because their degree of esterification was greater than 50%. These types ofpectin determine the mechanism for gel formation. On the other hand, jackfruit pectin was lower the percentage and it was a LM pectin (Bhat *et al*., 2014).

###

### 4.2.8 Solubility of dry pectin in cold and hot water

The solubility of dry pectin was observed in cold and hot water, given in the following table

**Table 4: Solubility of dry pectin in cold and hot water**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Guava**  | **Mango** | **Jackfruit** |
| Solubility of dry pectin in cold water | Insoluble | Insoluble | Insoluble |
| Solubility of dry pectin in hot water | Soluble | Soluble | Soluble |

###

### 4.2.9 Solubility of pectin solution in cold and hot alkali

The solubility of pectin solution was observed in cold and hot alkali, given in the following table

**Table 5: Solubility of pectin solution in cold and hot alkali**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Guava** | **Mango** | **Jackfruit** |
| Solubility of pectin solution in cold alkali | Forms yellow precipitate | Forms yellow precipitate | Forms yellow precipitate |
| Solubility of pectin in hot alkali | Soluble | Mixture dissolved and form a white precipitation | Mixture dissolved and form a white precipitation |

##

## 4.3 Chemical composition analysis of jellies

### 4.3.1 Moisture content determination

The moisture content of star fruit jelly, pineapple jelly and black berry jelly given below:

**Table 6: Moisture content of star fruit, pineapple and black berry jelly**

|  |  |
| --- | --- |
| **Jelly** | **Moisture content (%)** |
| **Star fruit jelly** | 21.73 |
| **Pineapple jelly** | 24.00 |
| **Black-berry jelly** | 25.12 |

###

### 4.3.2 Ash content

The ash content of star fruit jelly, pineapple jelly and black-berry jelly given in the following table:

**Table 7: Ash content of star fruit, pineapple and black berry jelly**

|  |  |
| --- | --- |
| **Jelly** | **Ash content (%)** |
| **Star fruit jelly** | 0.55 |
| **Pineapple jelly** | 0.28 |
| **Black-berry jelly** | 0.30 |

###

### 4.3.3 Total soluble solid (TSS)

The total soluble solid of star fruit jelly, pineapple jelly and black-berry jelly given in the following table

**Table 8: Total soluble solid (TSS) of star fruit, pineapple and black berry jelly**

|  |  |
| --- | --- |
| **Jelly** | **Total Soluble Solid ( TSS )** |
| **Star fruit jelly** | 67.00 |
| **Pineapple jelly** | 65.00 |
| **Black-berry jelly** | 63.00 |

###

### 4.3.4 Active acidity (pH)

The active acidity (pH)of star fruit jelly, pineapple jelly and black-berry jelly given in the following table:

**Table 9: Active acidity (pH) of star fruit, pineapple and black berry jelly**

|  |  |
| --- | --- |
| **Jelly** | **Active acidity (pH)** |
| **Star fruit jelly** | 3.2 |
| **Pineapple jelly** | 3.0 |
| **Black-berry jelly** | 2.6 |

##

## 4.4 Functional quality of jelly

### 4.4.1 Rate of setting and setting time

Guava peel and mango peel shows very fast setting and it took 20-30 minutes for proper setting of jelly. Jackfruit peel took 45-60 minutes for setting of jelly.

###

### 4.4.2 Consistency

Desirable consistency was observed from those three types of jelly made from pectin extracted from guava peel, mango peel and jackfruit peel.

###

### 4.4.3 Cloudiness

Cloudiness means the loss of transparent nature in jelly. But there was no cloudiness in these jellies. They were transparent.

##

# CHAPTER 5:DISCUSSION

In this study, pectin was extracted from guava peel, mango peel and jackfruit peel. The yield of pectin on dry basis were 13.44%, 8.26% and 5.94% from guava, mango and jackfruit peel. It was observed that the moisture content of jackfruit pectin was higher than the guava and mango pectin. But the other parameters such as ash, anhydrouronic acid content, methoxyl content, degree of esterification and equivalent weight of jackfruit pectin was lower than guava and mango pectin. The color of guava, mango and jackfruit pectin color were whitish to light brown, grayish to light brown and light brown. Respectively, with these pectin three different types of jelly were prepared, these were star fruit jelly, pineapple jelly and black-berry jelly. Star fruit jelly was prepared by guava pectin, pineapple jelly was prepared by mango pectin and black-berry jelly was prepared by jackfruit pectin. The functional quality of jellies was observed. All jellies were transparent, gel like consistency were observed. And the setting time of gel were faster in the star fruit and mango jelly, lower in the black-berry jelly.

From the above study, it was noted that, jelly prepared from guava peel and mango peel pectin were good than jackfruit pectin.

#

# CHAPTER 6: CONCLUSION

Every year our country produce lots of fruits which eventually give a large amount of fruit wastes. From this study, we could say fruit wastes like guava peel, mango peel and jackfruit peel are very rich in pectin. Pectin extracted from these fruit wastes are quite a bit similar to commercially available pectin. Every year, food industries as well as pharmaceuticals company import pectin for different uses. If we can extract pectin in our country from fruit wastes like guava peel, mango peel and jackfruit peel, it will be economically viable for industries and as well as will be eco-friendly.

#

#

# CHAPTER 7: RECOMMENDATION AND FUTIRE PERSPECTIVE

Recommendations of this study are-

* Sample (Fruit peel) should be collected in a clean sampling bag and kept in a freezer to prevent microbial attack.
* Over riped fruit peel cannot be used.
* During hydrolysis of peel, the pH range should be 1.2-2.6.

Future perspectives are-

* By using this method, we can achieve good quality and quantity of pectin.
* Seasonally available low cost fruits can be utilized in this way.
* Furits waste (Fuit peel) will not create environmental pollution.
* It will reduce waste load in the processing industries.
* It is a low economic extraction method.

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This is Dipika Majumder; Daughter of Dilip Majumder and Chandra Majumder. She has passed the Secondary School Certificate Examinations in 2006 followed by Higher Secondary Certificate Examination in 2008. She obtained her Food Science and Technology Degree in 2013 (held in 2014) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, CVASU.